The Journal of Infectious Diseases

MAJOR ARTICLE

A Genome-Wide Functional Genomics Approach Identifies Susceptibility Pathways to Fungal Bloodstream Infection in Humans

Martin Jaeger,[1,a] Vasiliki Matzaraki,[1,2,a] Raúl Aguirre-Gamboa,[2,a] Mark S. Gresnigt,[1] Xiaojing Chu,[2] Melissa D. Johnson,[3] Marije Oosting,[1] Sanne P. Smeekens,[1] Sebo Withoff,[2] Iris Jonkers,[2] John R. Perfect,[3] Frank L. van de Veerdonk,[1] Bart-Jan Kullberg,[1] Leo A. B. Joosten,[1] Yang Li,[2] Cisca Wijmenga,[2,4] Mihai G. Netea,[1,5] and Vinod Kumar[1,2]

[1]Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands; [2] University of Groningen, University Medical Center Groningen, Department of Genetics, the Netherlands; [3]Division of Infectious Diseases, Duke University Medical Center, Durham, North Carolina; [4] K.G. Jebsen Coeliac Disease Research Centre, Department of Immunology, University of Oslo, Norway; [5] Human Genomics Laboratory, Craiova University of Medicine and Pharmacy, Craiova, Romania

Background. Candidemia, one of the most common causes of fungal bloodstream infection, leads to mortality rates up to 40% in affected patients. Understanding genetic mechanisms for differential susceptibility to candidemia may aid in designing host-directed therapies.

Methods. We performed the first genome-wide association study on candidemia, and we integrated these data with variants that affect cytokines in different cellular systems stimulated with *Candida albicans*.

Results. We observed strong association between candidemia and a variant, rs8028958, that significantly affects the expression levels of *PLA2G4B* in blood. We found that up to 35% of the susceptibility loci affect in vitro cytokine production in response to *Candida*. Furthermore, potential causal genes located within these loci are enriched for lipid and arachidonic acid metabolism. Using an independent cohort, we also showed that the numbers of risk alleles at these loci are negatively correlated with reactive oxygen species and interleukin-6 levels in response to *Candida*. Finally, there was a significant correlation between susceptibility and allelic scores based on 16 independent candidemia-associated single-nucleotide polymorphisms that affect monocyte-derived cytokines, but not with T cell-derived cytokines.

Conclusions. Our results prioritize the disturbed lipid homeostasis and oxidative stress as potential mechanisms that affect monocyte-derived cytokines to influence susceptibility to candidemia.

Keywords. arachidonic acid metabolism; *C albicans*; cytokine-QTLs; genetic variants; *PLA2G4B*.

Invasive fungal infections are an increasingly significant cause of sepsis in critically ill and immunocompromised patients, and they are associated with high morbidity and mortality. *Candida albicans* is the most commonly encountered *Candida* species associated with nosocomial invasive candidiasis globally and is mainly part of the normal gut flora [1, 2]. *Candida albicans* causes both invasive and mucosal infections. It is a dimorphic fungus because it grows both as yeast and filamentous cells, and it causes approximately 250 000 new systemic infections on a yearly basis, leading to more than 50 000 deaths [3, 4].

Both innate and adaptive immune mechanisms are crucial for an effective host defense against fungal pathogens. One of the defense mechanisms used by phagocytes is the use of reactive oxygen species (ROS) against various pathogens, including fungi [5]. Cytokines play a critical role during anti-*Candida* host immune defense, and recombinant cytokines have been proposed as adjunctive therapy in systemic *Candida* infection [6–9]. Therefore, identifying genetic loci that influence susceptibility to systemic *Candida* infections by modulating ROS production as well as cytokine levels will help us understand what drives susceptibility, a crucial step for the design of novel preventive and therapeutic strategies.

In the present study, we performed the first genome-wide association study (GWAS) of candidemia on the largest patient cohort published to date consisting of 178 candidemia patients and 175 case-matched controls of European ancestry. By making use of cytokine-quantitative trait loci (cQTL) datasets generated from our previous studies from the Human Functional Genomics Project (HFGP) consortium [10, 11], we assessed the impact of genetic polymorphisms on the cytokine production capacity of peripheral blood mononuclear cells (PBMCs), whole blood, and

Received 28 January 2019; editorial decision 18 April 2019; accepted 23 April 2019; published online April 24, 2019.

Correspondence: Vinod Kumar PhD, Department of Genetics CB 50, University Medical Center, Groningen, P.O. Box 30 001, 9700 RB Groningen, The Netherlands (v.kumar@umcg.nl).

[a]M. J., V. M., and R. A.-G. contributed equally to the manuscript.

The Journal of Infectious Diseases® 2019;220:862–72

DOI: 10.1093/infdis/jiz206

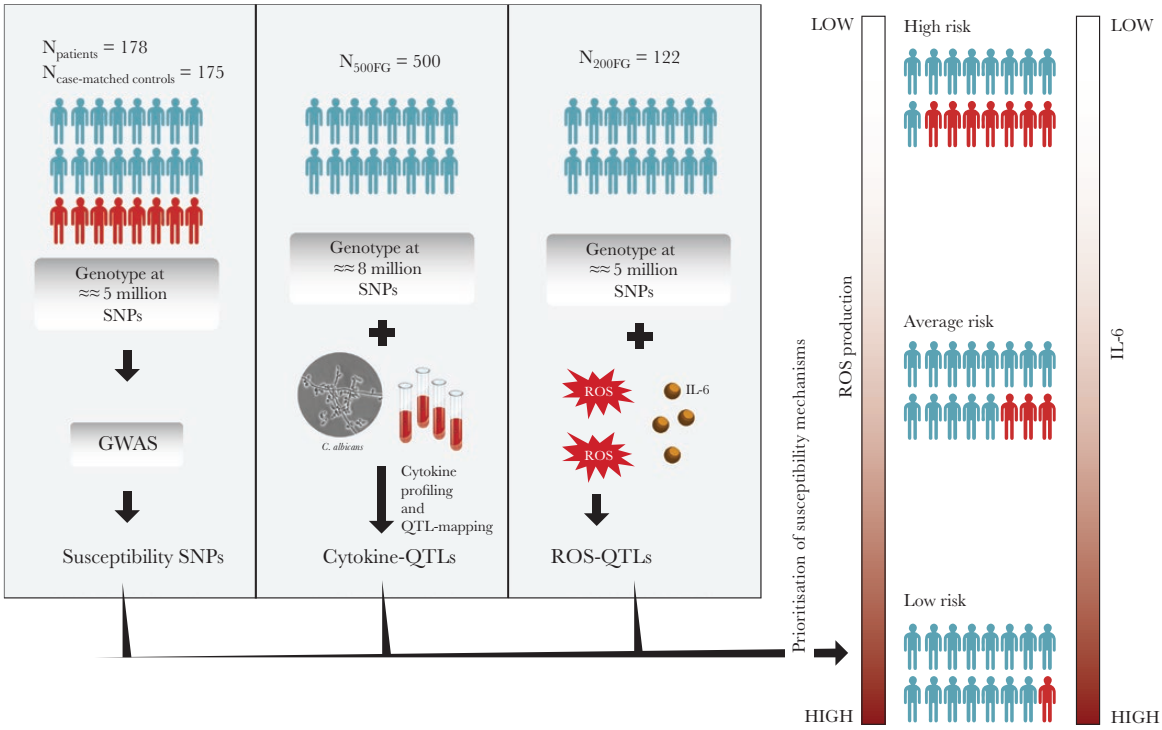

Figure 1. Study overview. We performed a genome-wide association study (GWAS) to test the association of genetic variants to candidemia susceptibility by using 178 patients and 175 case-matched controls. To identify whether susceptibility variants influence cytokine levels in response to *Candida albicans*, we first collected blood samples from 500 healthy individuals (500FG cohort), isolated their DNA, and genotyped it. Whole blood or peripheral blood mononuclear cells (PBMCs) or monocyte-derived macrophages were also isolated to perform a series of stimulation experiments with *C albicans* to profile cytokines released in the 3 cell systems. Using the genotype information and cytokine measurements, we mapped *Candida*-response cytokine quantitative trait loci (QTLs) in the 500FG cohort. Finally, we generated genotype data and measured reactive oxygen species (ROS) production from *Candida*-stimulated PBMCs isolated from an independent cohort of 122 healthy volunteers (200FG cohort). In addition, we profiled cytokine interleukin (IL)-6 produced from PBMCs isolated from a subgroup of 200FG cohort (n = 61). Taken all datasets, we showed that the numbers of risk alleles at candidemia loci, which influence cytokine levels in response to *Candida*, are negatively correlated with ROS production in response to *Candida*. Finally, ROS production showed a positive correlation with IL-6 measurements in 200FG. SNPs, single-nucleotide polymorphisms.

monocyte-derived macrophages in response to *C albicans* yeast or hyphae, in 500 healthy volunteers [10]. By integrating these cQTLs with the candidemia susceptibility data (Figure 1), we show that 35% of the independent susceptibility loci influence cytokine levels in response to *C albicans*, with several genes from these loci being known to be involved in lipid and arachidonic acid (AA) metabolic pathways. Furthermore, given that AA metabolism plays a role in the generation of ROS in various cell types [12], we show that the numbers of risk alleles at these loci are negatively correlated with ROS production in response to *Candida*, which is positively correlated with interleukin (IL)-6 levels, using an independent cohort of healthy volunteers. Taken together, our results suggest that increased risk to candidemia can be partly explained by a disturbed lipid homeostasis, which results in modulation of ROS and proinflammatory cytokines, which would ultimately lead to increased susceptibility to candidemia.

METHODS

Study Populations

Population-Based Cohorts

The study was performed using 2 independent population-based cohorts, 500FG and 200FG, composed of healthy individuals of Western European ancestry from the HFGP (www.humanfunctionalgenomics.org). For further description of these cohorts and ethics approval statements, see Supplementary Methods.

Candidemia Cohort

To identify single-nucleotide polymorphisms (SNPs) associated with candidemia susceptibility, we performed the first GWAS analysis of a well described candidemia cohort with corresponding case-matched controls [13]. In total, 178 candidemia cases and 175 case-matched controls of European ancestry were tested for disease association. The demographic and clinical characteristics of the candidemia cohort have been described previously [13].

Genotyping of the 500FG Cohort

Deoxyribonucleic acid (DNA) obtained from the 500FG cohort was genotyped using the commercially available Illumina HumanOmniExpressExome-8 v1.0 SNP chip. Genotype calling, quality control, and imputation were previously described elsewhere and in the Supplementary Methods [10].

Genotyping, Quality Control, and Imputation of the Candidemia Cohort

Isolated DNA obtained from case and control samples was genotyped using the commercially available SNP chips, HumanCoreExome-12 v1.0, and HumanCoreExome-24 v1.0 BeadChip from Illumina (https://www.illumina.com). Genotype calling, quality control, and imputation are described in Supplementary Methods.

Genome-Wide Case-Control Association Analysis

The associations between the genome-wide variants and candidemia susceptibility were tested by Fisher's exact test using PLINK v1.9 (www.cog-genomics.org/plink/1.9/) [14]. A *P* value significance threshold of $<5.0 \times 10^{-8}$ was set to call genome-wide significant associations. We considered SNPs with a *P* value between 9.99×10^{-5} and 5×10^{-8} as variants showing a suggestive association with candidemia susceptibility.

Cell Collection and *Candida* Stimulation Experiments

For the cytokine profiling, PBMCs, monocyte-derived macrophages and whole blood were collected and stimulated with heat-killed *C albicans* yeast (strain ATCC MYA-3573, UC 820) or hyphae in a concentration of 10^6 colony-forming units (CFU)/mL as previously described [10]. To identify the transcriptome upon *Candida* stimulation, 5×10^5 PBMCs isolated from 8 healthy volunteers were incubated with 1×10^6/mL heat-killed *C albicans* (UC 820) and Roswell Park Memorial Institute (RPMI) culture medium as a control for 4 and 24 hours, as previously described [15]. For further details on the experiments, see Supplementary Methods.

Analysis of Ribonucleic Acid Sequencing Reads

Ribonucleic acid (RNA) sequencing analysis of the data generated from *Candida*-stimulated PBMCs for 4 and 24 hours was previously described (see Supplementary Methods) [15].

Cytokine-Quantitative Trait Loci Mapping

We made use of cQTL datasets generated from our previous studies from the HFGP consortium, which are publicly available at www.humanfunctionalgenomics.org [10]. To correct for multiple testing, we used Bonferroni and Benjamini-Hochberg approaches for the 26 susceptibility variants located in 24 independent loci. We considered $P < 5 \times 10^{-8}$ to be the threshold for significant cQTLs. *Candida albicans* response QTLs ($P < .05$) were intersected with SNPs associated with susceptibility to candidemia showing a *P* value between 9.99×10^{-5} and 5×10^{-8} to identify cQTLs associated with susceptibility.

Peripheral Blood Mononuclear Cell Fungal Killing Assays

Human PBMCs isolated from healthy volunteers were plated in 96 round-bottom plates (5×10^5 cells per well). After 24 hours of incubation, 25 nM of either nontargeting small interfering RNA (siRNA) control pool (D-001810-10-05) or PLA2G4B (L-187552-01-0005) targeting siRNA (SMARTpool, Thermo Fischer Scientific) were added for 24 hours. After the transfection period, 1×10^6/mL live *Candida* was added for 24 hours. After incubation, the well contents were serially diluted in sterile water and plated on Sabouraud agar for counting of CFUs after 24 hours of incubation. Experiments were performed 3 times with 2 donors (N = 6) in each individual experiment. The serial dilutions were plated in duplicate, and the mean value of both measurements was used for the analysis: $P < 0.05$ = *, $P < .01$ = ** and $P < .001$ = ***.

Reactive Oxygen Species Production

Reactive oxygen species were measured in PBMCs of healthy volunteers from the 200FG cohort. Induction of ROS was measured by oxidation of luminol (5-amino-2,3-dihydro-1,4-phtalazinedione). The PBMCs (5×10^5) of healthy volunteers were resuspended in Hanks'Balanced Salt Solution and put in dark 96-well plates. Cells were exposed to 1×10^6/mL *C albicans* yeast (UC820, heat killed) together with 20 µL of 1 mM luminol (final concentration 50 µM). Chemiluminescence was measured in BioTek Synergy HTreader at 37°C for 1 hour with intervals of 2.23 minutes.

Quantitative Trait Loci Mapping of Reactive Oxygen Species Production

We selected 137 healthy individuals from 200FG for whom both genotype and measurements of ROS production were available. We applied a $\log_2$ transformation on raw measurements of ROS production. The correlation between the transformed phenotype with genotypes was tested by a linear regression model using age and gender as covariables, using the R-package Matrix-eQTL. We considered $P < .05$ to be the threshold for suggestive QTLs.

Constructing Allelic Scores

We constructed allelic risk scores for candidemia susceptibility using 122 individuals from the 200FG cohort for whom we have genotypes and ROS measurements available. We calculated an allelic score per individual as the sum of the number of minor alleles across all susceptibility variants multiplied by the $\log_2$ of the odds ratio (OR) of that particular SNP for candidemia susceptibility. Allelic scores were calculated using PLINK v1.9 (www.cog-genomics.org/plink/1.9/) [14].

Correlation Analyses

All correlations were tested by non-parametric Spearman's rank correlation test using R version 3.3.2 (available at http://www.R-project.org). Both ROS and IL-6 measurements were $\log_2$ transformed. Measurements of IL-6 levels from *Candida*-stimulated PBMCs were available for 61 individuals from the 200FG cohort measured with enzyme-linked immunosorbent assay in 2017. $P < .05$ was considered as the threshold for significance.

Pathway Enrichment Analysis

We performed pathway enrichment analysis using a novel tool called *Pascal* (Pathway scoring algorithm). We applied *Pascal* to

define gene-level *P* values for all genetic loci that showed association to candidemia susceptibility with a *P* value of $<9.99 \times 10^{-4}$ using default parameters. The *Pascal* tool is described in detail elsewhere [16]. Results reported in this paper are based on the empirical *P* values; similar results were obtained using the χ^2 statistic.

RESULTS

Candidemia Genome-Wide Association Study Identifies a Susceptibility Locus on Chromosome 15

To identify genetic variants associated with candidemia susceptibility, we genotyped 178 well described candidemia patients and 175 case-matched controls of European ancestry [13] on a genome-wide SNP platform and imputed the genotype data using the Human Reference Consortium [17]. After quality control filtering per SNP and sample (Figure S1A and B), we obtained 161 patients and 152 case-matched controls for which we had genotype information on 5 326 313 SNPs, which were tested for disease association. This GWAS identified 235 SNPs in 69 independent loci that show suggestive disease associations ($P < 9.99 \times 10^{-5}$). Among these loci, an SNP on chromosome 15, rs8028958, showed the strongest association with susceptibility to candidemia ($P = 7.97 \times 10^{-8}$, OR = 0.4002, imputation quality score $R^2 = 0.97$) (Figure 2A) and is located within the intronic region of the *SPTBN5* gene (Figure 2B).

Prioritization of *PLA2G4B* as Plausible Causal Gene

To identify potential causal genes affected by rs8028958, we made use of expression-QTL (eQTL) datasets derived from whole blood of healthy donors. In addition, we tested the expression levels of all genes located within a 500-kilobase *cis*-window of all 69 candidemia variants with suggestive association ($P < 9.99 \times 10^{-5}$) in PBMCs stimulated with *C albicans* yeast for 4 and 24 hours. For eQTL analysis, we made use of the largest *cis*- and *trans*-expression QTL study in blood from 31 684 individuals through the eQTLGen Consortium [18]. The results from this eQTL mapping at rs8028958 identified a significant effect on expression levels of *PLA2G4B* gene ($P = 7.9 \times 10^{-158}$). In addition, we observed significant downregulation of *PLA2G4B* expression ($P = 1.02 \times 10^{-13}$) upon *C albicans* stimulation for 24 hours in PBMCs, which helped to prioritize *PLA2G4B* as a plausible candidemia susceptibility gene (Figure S2A). Furthermore, publicly available transcriptomic data from PBMCs isolated from patients with chronic mucocutaneous candidiasis (CMC), which were stimulated with RPMI and heat-killed *C albicans*, showed a trend of decreased expression of *PLA2G4B* in response to *C albicans* compared with RPMI control medium (Figure S2B) [19]. The CMC patients have increased susceptibility to mucosal fungal infections and often carry gain-of-function mutations in STAT1 [20, 21].

PLA2G4B (cytosolic phospholipase A_2 group IVB; also termed cPLA2-beta) encodes a member of the cytosolic phospholipase A_2 protein family [22]. It is interesting to note that the other member of this family, cPLA2-alpha, has also been shown to modulate inflammation and immune responses to *Candida*, which suggests a critical role of phospholipid metabolism pathway in candidemia [23]. Therefore, we conducted specific *PLA2G4B* siRNA or control siRNA experiments in human PBMCs to silence the gene before stimulation with *C albicans*. Although it was not statistically significant ($P = .08$), we observed that targeted silencing of *PLA2G4B* led to an increased trend of

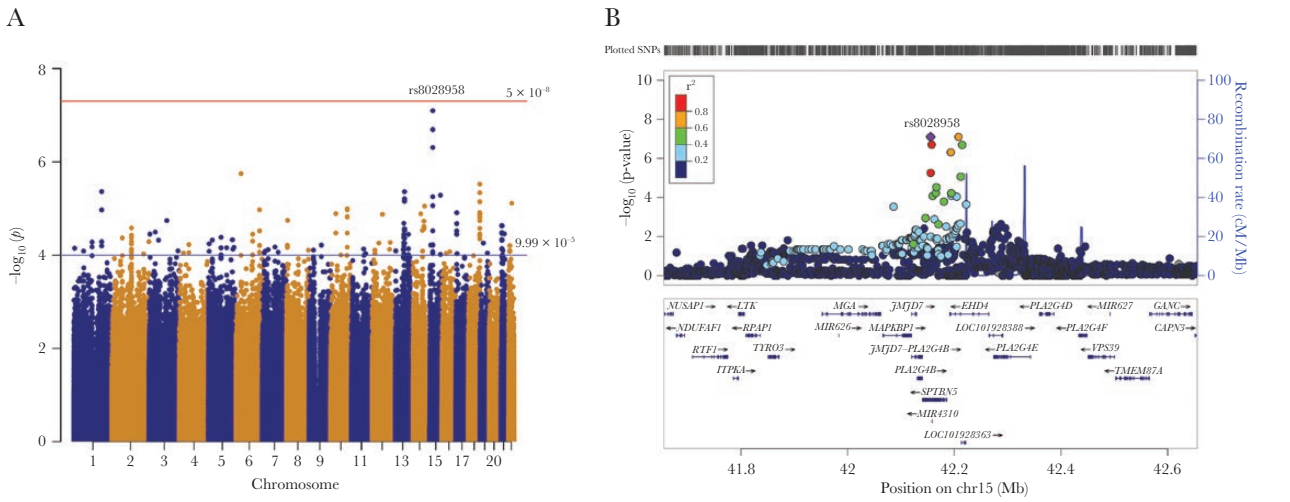

Figure 2. *PLA2G4B* locus is associated with candidemia susceptibility. (A) Manhattan plot of single-nucleotide polymorphisms (SNPs) associated with candidemia susceptibility identified by the genome-wide association study (GWAS). The GWAS revealed a locus strongly associated with susceptibility to candidemia at chromosome 15 ($P = 7.97 \times 10^{-8}$, odds ratio = 0.4002). The y-axis represents $-\log_{10}$ *P* values of SNPs. The x-axis shows chromosomal positions. The blue line is the suggestive threshold for association ($P < 9.99 \times 10^{-5}$). (B) The regional association plot for the candidemia-associated SNP, rs8028958 (purple diamond), which is located within the intronic region of the *SPTBN5* gene at chromosome 15. Each dot represents an SNP, and the linkage disequilibrium of neighboring SNPs with the top SNP is color coded. The y-axis represents $-\log_{10}$ *P* values of SNPs. The x-axis shows chromosomal positions.

PBMCs to kill *C albicans* yeast (Figure S2C), suggesting a functional role for *PLA2G4B* in host defense against *C albicans*.

Candidemia Genetic Loci Are Enriched for Phospholipid Metabolism

Given the function of *PLA2G4B* in phospholipid metabolism, we hypothesized that genetic loci that show suggestive association with candidemia ($P < 9.99 \times 10^{-4}$) are also involved in phospholipid metabolism. To test this, we ran a pathway enrichment analysis using the *Pascal* tool. *Pascal* first allows mapping individual SNPs to genes, and their association *P* values are combined into gene scores (Supplementary Table S1); second, genes are grouped into pathways, and their gene scores are combined into pathway scores (Supplementary Table S2) [16]. We found a significant enrichment of candidemia genes in alpha-Linolenic acid ($P = .006$), linoleic acid ($P = .007$), phospholipid ($P = .007$), and AA metabolism ($P = .03$) (Figure 3, Supplementary Table S2). Arachidonic acid is a polyunsaturated fatty acid present in the phospholipids of cell membranes, conferring them with fluidity and flexibility, and alpha-Linolenic and linoleic acids are major dietary polyunsaturated fatty acids in the Western diet and are metabolic precursors to AA [24, 25].

Next, we prioritized potential causal genes from these candidemia loci ($P < 9.99 \times 10^{-5}$) by using 3 different approaches (Supplementary Methods). Our 3 approaches prioritized 129 candidate genes (Supplementary Table 3) located in 69 susceptibility loci ($P < 9.99 \times 10^{-5}$). Of note, 19 of these 131 prioritized genes are known to be involved in lipid metabolic processes and AA metabolism. In addition to the *PLA2G4B* gene, these loci encompassed genes such as *SLC16A11*, *GGT5*, *GSTT1*, *AGPAT5*, and *AGPAT1*, which are known to be involved in lipid metabolism. Genes, other than the PLA_2 family, such as *ALOX12B*, *ALOXE3*, and *ALOX15B* encode members of the family of enzymes called arachidonate lipoxygenases, which are known to metabolize AA to different metabolites. Other interesting genes relevant to phospholipid synthesis and metabolism are *TTC7B* and *HSD17B4* genes [26, 27]. Finally, genes in the candidemia-associated locus rs4369966 on chromosome 22 encoding Apolipoprotein L (APOL) proteins, which belong to the high-density lipoprotein family, were found to be significantly differentially expressed after 4-hour *Candida* stimulation (*APOL1*, *APOL6*) and 24-hour *Candida* stimulation (*APOL1*, *APOL2*, *APOL3*, *APOL4*, *APOL6*). The APOL proteins are known to play a central role in cholesterol transport. Taken

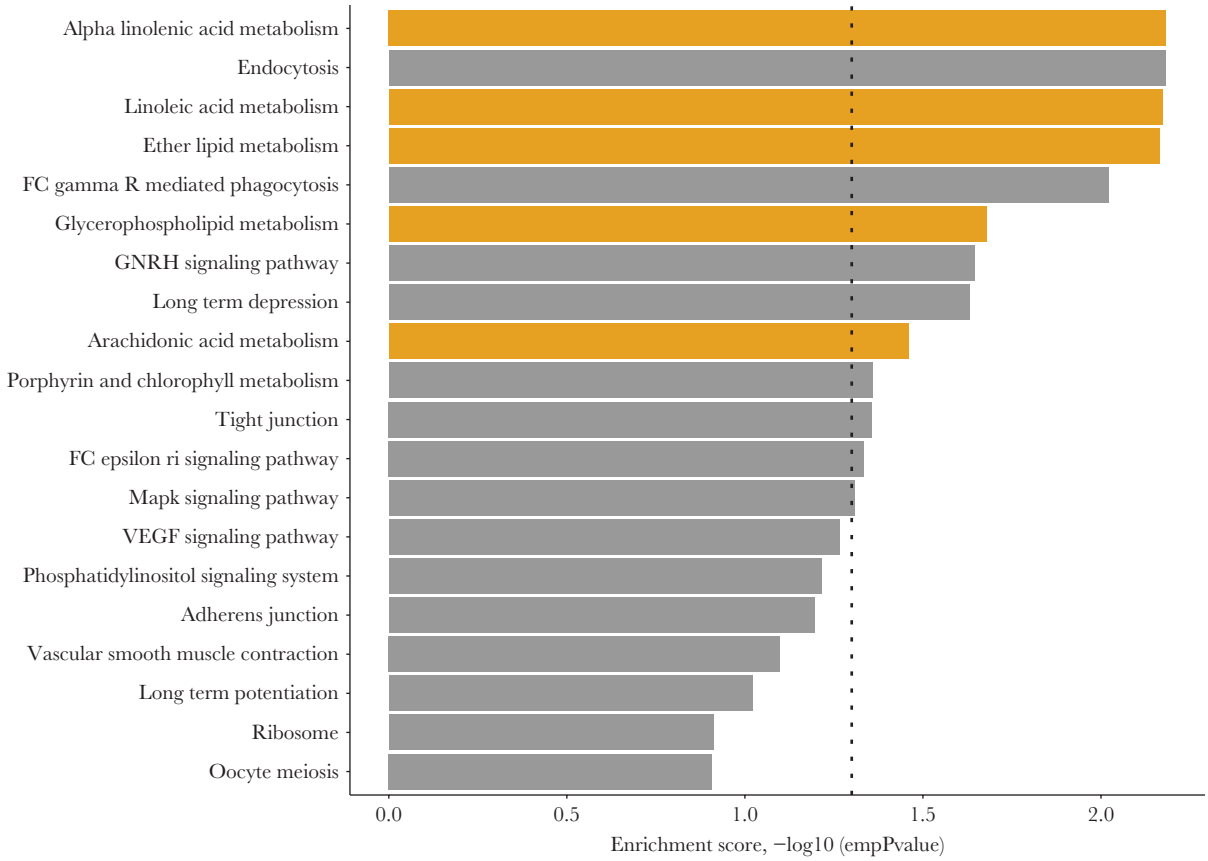

Figure 3. The pathway enrichment analysis of genes from candidemia loci ($P < 9.99 \times 10^{-4}$) showed a significant enrichment in alpha-linolenic acid ($P = .006$), lipid ($P = .006$), and arachidonic acid metabolism ($P = .03$). KEGG was used as a database resource of the molecular pathways. The empirical *P* values are presented, and the dashed black line corresponds to a *P* threshold of significance .05.

together, our gene prioritization pinpointed several genes that are known to be involved in lipid and AA metabolic processes, suggesting a crucial role for these metabolic processes in regulating inflammation and susceptibility to candidemia.

Candidemia Single-Nucleotide Polymorphisms Affecting Phospholipid Metabolism Regulate Cytokine Levels in Response to *Candida albicans*

It is known that the phospholipid metabolism influences inflammation in response to infections [28–30]. Therefore, we hypothesized that candidemia-associated SNPs, together with phospholipid metabolism, may also affect cytokine production in response to *Candida* infection. We therefore tested whether SNPs associated with susceptibility to candidemia ($P < 9.99 \times 10^{-5}$) influence cytokine levels in response to *C albicans* yeast or hyphae using the results of the previous cQTL mapping [10]. We found 26 SNPs from 24 independent candidemia-susceptibility loci that modulate cytokine levels upon *C albicans* yeast or hyphae stimulation in any of the cell systems (PBMCs, whole blood, and macrophages) (Table 1, Supplementary Table 4). Of note, the candidemia-associated SNP on chromosome 15, rs12593397 (missense variant), is correlated (D' = 0.94, $r^2 = 0.49$) with rs8028958 in a European population, which showed the strongest association to candidemia susceptibility (Supplementary Table 5). Genes within 7 independent candidemia-associated loci are known to be involved in lipid and AA metabolism pathways (Table 1). In addition, this analysis also identified important innate immune genes such as *NOD2* and *IL21R* for candidemia susceptibility. These observations led us to the hypothesis that susceptibility to candidemia can be partly explained by defects in phospholipid metabolism and, subsequently, to AA metabolic processes, which could ultimately lead to dysregulated cytokine responses.

Genetic Basis for Interplay Among Phospholipid Metabolism, Cytokine Responses, and Reactive Oxygen Species Production in Candidemia

Given that ROS production is one of the downstream products of AA metabolism, we tested whether the candidemia SNPs ($P = 9.99 \times 10^{-5}$) have an impact on ROS production in response to *C albicans* [31, 32]. For this, we generated genotypes and measurements of ROS production in PBMCs of 122 healthy volunteers from a 200FG cohort. Due to the small cohort size, only the candidemia-associated SNP, rs2725008, on chromosome 8 was found to significantly influence ROS production ($P = .007$) (Supplementary Table S6). Five additional candidemia-associated SNPs influenced ROS production with a P value of nominal significance ($P < .05$) (Supplementary Table S6). Next, to examine the overall impact of candidemia susceptibility SNPs on ROS production, we constructed an allelic risk score for candidemia susceptibility based on the 24 independent susceptibility variants that affect cytokine levels and correlated with ROS levels (Figure 4A). We observed a strong negative correlation between the abundance of ROS and allelic scores ($P = .0065$, r = −0.24). This correlation remained significant only when calculating allelic scores based on 16 independent candidemia-associated SNPs that affect only monocyte-derived cytokine levels (IL-6, IL-1β, and tumor necrosis factor [TNF]α) ($P = .00063$, r = −0.31) (Figure 4B). The correlation did not remain significant when using 12 independent candidemia-associated variant SNPs that affect T cell-derived cytokines (IL-17, IL-22, and interferon [IFN]γ) (Figure 4C). No significant correlations observed when allelic scores constructed as the total number of risk alleles either from 49 susceptibility loci ($P = 0.57$, r = −0.052) (excluding those that influence *Candida*-induced cytokines) (Figure S3A) or 69 susceptibility SNPs ($P < 9.99 \times 10^{-5}$), including those that influence *Candida*-induced cytokines ($P = 0.15$, r = −0.13) (Figure S3B). Furthermore, we correlated the ROS measurements with the levels of the IL-6 produced in *Candida*-stimulated PBMCs. We observed a strong positive correlation between IL-6 and ROS levels (r = 0.27, $P = .036$) (Figure S4). Overall, these findings indicate that candidemia-associated loci have a functional role in the anti-*Candid*a defense mechanism by influencing cytokine and ROS production upon *Candida* infection.

DISCUSSION

In a given at-risk population, not all patients develop infections, which indicates that there is a strong genetic influence on individual susceptibility to different infections [33]. Genetic association studies that identify risk genes for systemic fungal infections are extremely challenging because of the difficulty of enrolling large numbers of patients and of using appropriate controls, such as case-matched controls. To circumvent these challenges, we combined traditional GWAS strategy with systems biology approaches. By testing whether SNPs associated with susceptibility to candidemia influences cytokine levels and ROS production in response to *C albicans* in an unbiased manner, we prioritize critical genes and pathways for candidemia susceptibility.

We identified a novel strong genetic association of candidemia with polymorphisms in *PLA2G4B*. It has been suggested that PLA2G4B-F enzymes may control phospholipid and/or arachidonate metabolism in a tissue-specific manner [34]. However, the role of *PLA2G4B* has never been studied in the context of infectious diseases. We observed that specific downregulation of *PLA2G4B* using siRNA resulted in a tendency to improve the candidacidal properties of PBMCs, suggesting a role of this gene in the host response against *C albicans*. Of note, genes from other susceptibility loci showed a significant enrichment in alpha-Linolenic acid and linoleic acid (metabolic precursors of AA), phospholipid, as well as AA metabolism.

Furthermore, we demonstrated the moderate impact of *Candida*-induced cQTLs on susceptibility to candidemia. As shown in Table 1, there are 24 independent cQTLs that

Table 1. SNPs Modulating Cytokines in Response to *C. albicans* Show Strong association with Susceptibility ($P < 9.99 \times 10^{-5}$).

		P value							
Chr	SNP	Susceptibility	cQTL	OR	Cytokine	Stimulant	Cell system	Time	*Gene(s)*
1	rs11588087	4.34×10^{-6}	0.014	3.07	IL-1β	Yeast	PBMCs	24h	*GLUL(a), RGS16(a)*
2	rs34089869	4.26×10^{-5}	0.019	0.35 IFNγ	Yeast		PBMCs	7days	
2	rs62159917	3.74×10^{-5}	0.049	0.36	TNF-α	Yeast	Macrophages	24h	
3	rs12493413	3.54×10^{-5}	0.021	0.49	IL-22	Hyphae	PBMCs	7days	*ARL8B*(a), *BHLHE40* (a), *SUMF1(g)*
3	rs9879846	4.79×10^{-5}	0.036	0.44	IFNγ	Yeast	Whole blood	48h	*C3orf14(a,c)*
5	rs78969277	4.69×10^{-5}	0.026	3.29	TNF-α	Yeast	Macrophages	24h	*ADAM19(a,b), HAVCR2(a), NIPAL4(a), ITK(c)*
5	rs4895365	6.94×10^{-5}	0.019	0.46	IL-1β	Hyphae	PBMCs	24h	*HSD17B4(c,g), TNFAIP8(b,g), DMXL1(c)*
5	rs6872016	4.76×10^{-5}	0.015	0.39	IL-1β	Yeast	Whole blood	48h	
6	rs11155859	1.06×10^{-5}	0.044	0.48	IFNγ	Yeast	PBMCs	7days	*SYNE1(c)*
6	rs11155859	1.06×10^{-5}	0.031	0.48	IL-6	Yeast	Macrophages	24h	*SYNE1(c)*
6	rs11155859	1.06×10^{-5}	0.048	0.48	IL-6	Yeast	Whole blood	48h	*SYNE1(c)*
7	rs3807184	7.63×10^{-5}	0.013	0.45	IL-17	Hyphae	PBMCs	7days	*NME8(a,c,e), EPDR1(b,c,d),GPR141(c),TXNDC3(c), STARD3NL(g)*
7	rs3807184	7.63×10^{-5}	0.036	0.45	IL-22	Hyphae	PBMCs	7days	*NME8(a,c,e), EPDR1(b,c,d),GPR141(c),TXNDC3(c), STARD3NL(g)*
8	rs2725008	6.17×10^{-5}	0.015	0.43	IL-6	Yeast	Macrophages	24h	
8	rs2725008	6.17×10^{-5}	0.015	0.43	TNF-α	Yeast	Macrophages	24h	
10	rs10884489	1.02×10^{-5}	0.032	0.48	TNF-α	Yeast	Macrophages	24h	
11	rs1945666	7.37×10^{-5}	0.038	1.99	IL-17	Yeast	PBMCs	7days	
11	rs1945666	7.37×10^{-5}	0.038	1.99	TNF-α	Yeast	Macrophages	24h	
12	rs7306373	1.33×10^{-5}	0.034	0.47	IFNγ	Hyphae	PBMCs	7days	*CPM(a,b), LYZ(a,b)*
13	rs9530375	5.45×10^{-5}	0.049	2.329	IFNγ	Yeas	PBMCs	7days	*PLEKHA1(d)*
13	rs7322708	2.08×10^{-5}	0.026	2.34	IL-22	Hyphae	PBMCs	7days	*CMIP(d),HSPA9(d), PLEKHA1(d), PPP1R8(d)*
13	rs6563046	4.35×10^{-6}	0.001	2.12	IL-6	Yeast	Macrophages	24h	*RNF219(c)*
13	rs6563046	4.35×10^{-6}	0.002	2.12	TNF-α	Yeast	Macrophages	24h	*RNF219(c)*
13	rs7337751	7.61×10^{-5}	0.017	2.05	TNF-α	Yeast	Macrophages	24h	
14	rs71423384	1.29×10^{-5}	0.041	0.39	IL-1β	Hyphae	PBMCs	24h	*SLC8A3(a), KIAA0247, SLC10A1(g)*
15	rs12593397	3.02×10^{-5}	0.037	0.51	IFNγ	Yeast	Whole blood	48h	*EHD4(b,c,d), JMJD7-PLA2G4B(c,g),RPAP1(c), PLA2G4B(c,g), SPTBN5(c), RP11-23P13.6(c), RP11-107F6.3(c), JMJD7(c), MAPKBP1(c), MGA(c), CTD-2382E5.3(c), PLA2G4D(g), PLA2G4E*(g), *PLA2G4F(g)*
16	rs2189521	9.14×10^{-5}	0.048	0.52	IL-1β	Yeast	PBMCs	24h	*GTF3C1(c), IL21R(c)*
16	rs4785438	8.50×10^{-5}	0.039	0.53	IL-1β	Yeast	Whole blood	48h	*CARD15(c), NOD2(a,c,d), CYLD(c), NKD1(c), RP11-401P9.4(c), RP11-327F22.6(c)*
17	rs3027232	9.27×10^{-5}	0.019	0.47	IL-17	Yeast	PBMC	7days	*C17orf44(c), TMEM107(c), TMEM88(b), ALOX15B(a,g), ALOXE3(a,f,g), LINC00324(c), ALOX12B(g)*
17	rs3027232	9.27×10^{-5}	0.009	0.47	IL-22	Yeast	PBMCs	7days	*C17orf44(c), TMEM107(c), TMEM88(b), ALOX15B(a,g), ALOXE3(a,f,g), LINC00324(c), ALOX12B(g)*
17	rs3027232	9.27×10^{-5}	0.005	0.47	IFNγ	Yeast	Whole blood	48h	*C17orf44(c), TMEM107(c), TMEM88(b), ALOX15B(a,g), ALOXE3(a,f,g), LINC00324(c), ALOX12B(g)*

Table 1. Continued

		P value							
Chr	SNP	Susceptibility	cQTL	OR	Cytokine	Stimulant	Cell system	Time	*Gene(s)*
17	rs3027232	9.27×10^{-5}	0.046	0.47	IL-1β	Yeast	Whole blood	48h	*C17orf44(c), TMEM107(c), TMEM88(b), ALOX15B(ag), ALOXE3(a,f,g), LINC00324(c), ALOX12B(g)*
17	rs3027232	9.27×10^{-5}	0.036	0.47	IL-6	Yeast	Whole blood	48h	*C17orf44(c), TMEM107(c), TMEM88(b), ALOX15B(a,g), ALOXE3(a,f,g), LINC00324(c), ALOX12B(g)*
18	rs72987764	3.01×10^{-6}	0.042	0.38	IL-17	Yeast	PBMCs	7days	
18	rs72987756	2.65×10^{-5}	0.04	0.449	IL-6	Yeast	Macrophages	24h	
18	rs72987756	2.65×10^{-5}	0.03	0.449	TNF-α	Yeast	Macrophages	24h	
22	rs113413	8.04×10^{-5}	0.029	2.03	IL-17	Hyphae	PBMC	7days	*AP000350.4(c), AP000350.5(c), AP000350.6(c),AP000351.10* (c), *DDT(c,d), DDTL(c),GSTT1(c,d),KB-226F1.1(c),KB-226F1.2(c), MIF(d), GGT5(a,g), DDT22(c), UPB1(c),*

Abbreviations: Chr, chromosome; cQTL, cytokine quantitative trait loci; IFN, interferon; IL, interleukin; OR, odds ratio; PBMCs, peripheral blood mononuclear cells; SNP, single-nucleotide polymorphisms; TNF, tumor necrosis factor.

[a] Differentially-expressed genes after 24-hour *Candida* stimulation.

[b] Differentially-expressed genes after 4-hour *Candida* stimulation.

[c] Candidemia-associated SNPs or proxies ($r^2 > 0.8$) showed an eQTL effect in whole blood based on publicly available eQTL datasets [18,47,48].

[d] Candidemia-associated SNPs or proxies ($r^2 > 0.8$) showed an eQTL effect in monocytes [46,49].

[e] Candidemia-associated SNP in strong LD ($r^2 > 0.8$) with a synonymous variant.

[f] Candidemia-associated SNP is a missense variant.

[g] Gene is involved in lipid metabolism (Reactome).

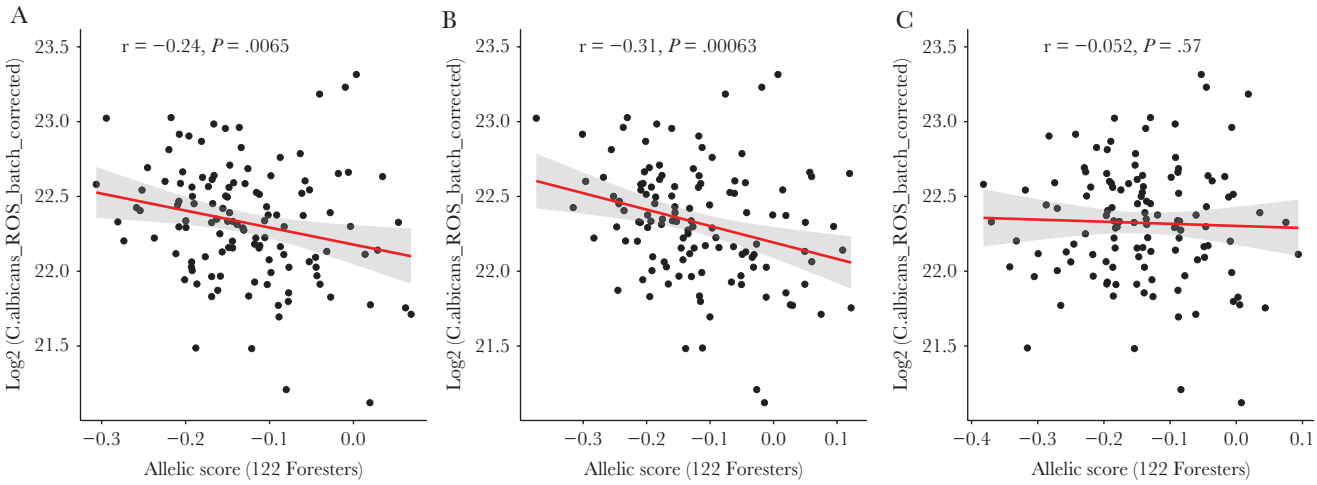

Figure 4. Candidemia-associated loci have a functional role in anti-*Candida* defense mechanism by influencing reactive oxygen species (ROS) production in response to *Candida albicans*. Scatterplots of significant correlations between ROS production and allelic scores constructed as the total number of risk alleles from (A) 24 susceptibility variants that influence cytokines and (B) 16 variants that only influence monocyte-derived cytokines (interleukin [IL]-6, IL-1β, tumor necrosis factor α) in response to either *C albicans* yeast or hyphae in either of the 3 cell systems (peripheral blood mononuclear cells, whole blood, and monocyte-derived cytokines). (C) Scatterplot of nonsignificant correlation between ROS production and allelic scores constructed as the total number of risk alleles from 12 susceptibility variants that only influence T-cell cytokines (IL-17, IL-22, and IFNγ) in response to either *C albicans* yeast or hyphae in either of the 3 cell systems. Allelic scores were weighted by odds ratio. Genotype and ROS production data were available for 122 individuals from the 200FG cohort. The (rank-based) Spearman correlation was calculated between ROS production and allelic scores. $P < .05$ was considered as the threshold for significance.

modulate candidemia susceptibility. Genes well known to be involved in lipid and AA metabolic processes were found in these loci. For instance, genes encoding for members of the lipoxygenase family, such as *ALOX15B*, *ALOXE3* and *ALOX12B*, in candidemia-associated locus rs3027232, are known to catalyze the attachment of molecular oxygen O_2 to polyunsaturated fatty acids, such as AA. It has been previously reported that a mutation in *ALOXE3* gene among nonbullous congenital ichthyosiform erythroderma patients predispose the patients to high risk of severe cutaneous fungal infections [35]. Many metabolic changes, including lipid synthesis, are shown to be important in regulating cytokine secretion to coordinate the immune response [36–38]. In mouse studies, hyperlipoproteinemia increased the susceptibility to systemic candidiasis due to an increased fungal outgrowth in their organs [39]. In human studies, infusion of lipoproteins enhanced the growth of *C albicans* in the plasma of volunteers [40], suggesting that hyperlipidemia have deleterious effects by enhancing the growth of *C albicans* in both species

One of the well known mechanisms of action of AA is triggering the generation of superoxides (O_2^-) in leukocytes, which act as microbicidal agents in phagocytes and a second messenger in many cell types [41, 42]. Thus, one would expect that ROS decrease or depletion would be associated with increased susceptibility to infection. Indeed, mice deficient in ROS production have been described to be more susceptible to different infections [5]. In addition, chronic granulomatous disease patients, who lack a functional NOX2 protein, show an increased susceptibility to various pathogens, including bacteria and fungi such as *Candida* and *Aspergillus* [43]. In this study, we observed that the numbers of risk alleles at candidemia susceptibility loci are negatively correlated with ROS production in response to *C albicans*. Of note, the correlation remained significant when using susceptibility variants that only influence monocyte-derived cytokines (IL-6, IL-1β, and TNFα), but not T cell-derived cytokines (IL-17, IL-22, and IFNγ). These data show that individuals with a higher genetic risk to candidemia susceptibility show a decreased ROS as well as cytokine production in response to *C albicans* infection. In the future, stratifying patients based on their genetic profiling would allow us to identify those at high-risk, who will benefit most from the treatment.

CONCLUSIONS

There are some limitations in this study. First, although it is difficult to gather large cohorts of patients, we need independent candidemia cohorts to replicate the genetic associations. Second, the publicly available eQTL datasets from whole blood used miss cell-type specific [44–46] or context-specific eQTLs upon *Candida* infection. Nevertheless, our approach to correlate the polygenic risk score for candidemia with ROS and cytokine levels in an independent cohort prioritized the disturbed lipid homeostasis and oxidative stress as potential genetic mechanisms that affect cytokine production to influence susceptibility to candidemia.

SUPPLEMENTARY DATA

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to

benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank all volunteers from the 500 and 200 Functional Genomics cohort of the Human Functional Genomics Project for participation in the study. We also thank K. McIntyre for editing the final text. Finally, we thank the UMCG Genomics Coordination center, the UG Center for Information Technology, and their sponsors BBMR-NL and Target for storage and compute infrastructure.

Financial support. This work was funded by the following: a research grant (2017) from the European Society of Clinical Microbiology and Infectious Diseases and a Radboudumc Hypatia Grant (2018) (to V. K.); a European Research Council (ERC) Consolidator Grant (FP/2007–2013/ERC Grant 2012-310372) and a Netherlands Organization for Scientific Research (NWO) Spinoza Prize Grant (NWO SPI 94-212; to M. G. N.); an ERC Advanced Grant (FP/2007–2013/ERC Grant 2012-322698) and an NWO Spinoza Prize Grant (NWO SPI 92-266; to C. W.); and a European Union Seventh Framework Program Grant (EU FP7) TANDEM Project (HEALTH-F3-2012-305279; to C. W. and V. K.). Y. L. and M. O. were supported by VENI Grants (863.13.011 and 016.176.006) from the NWO. M. D. J. was supported by a grant from the National Institutes of Health (AI-51537).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Guinea J. Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect **2014**; 20 (Suppl 6):5–10.
2. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med **2012**; 4:1–9.
3. Boyce KJ, Andrianopoulos A. Fungal dimorphism: the switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. FEMS Microbiol Rev **2015**; 39:797–811.
4. Campion EW, Kullberg BJ, Arendrup MC. Invasive candidiasis. N Engl J Med **2015**; 373:1445–1456.
5. Paiva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens? Antioxid Redox Signal **2014**; 20:1000–37.
6. van de Veerdonk FL, Kullberg BJ, Netea MG. Adjunctive immunotherapy with recombinant cytokines for the treatment of disseminated candidiasis. Clin Microbiol Infect **2012**; 18:112–9.
7. Armstrong-James D, Brown GD, Netea MG, et al. Immunotherapeutic approaches to treatment of fungal diseases. Lancet Infect Dis **2017**; 17:e393–402.
8. Delsing CE, Gresnigt MS, Leentjens J, et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. BMC Infect Dis **2014**; 14:166.
9. Buddingh EP, Leentjens J, van der Lugt J, et al. Interferon-gamma immunotherapy in a patient with refractory disseminated candidiasis. Pediatr Infect Dis J **2015**; 34:1391–4.
10. Li Y, Oosting M, Smeekens SP, et al. A functional genomics approach to understand variation in cytokine production in humans. Cell **2016**; 167:1099–110.e14.
11. Li Y, Oosting M, Deelen P, et al. Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. Nat Med **2016**; 22:952–60.
12. Cho KJ, Seo JM, Kim JH. Bioactive lipoxygenase metabolites stimulation of NADPH oxidases and reactive oxygen species. Mol Cells **2011**; 32:1–5.
13. Kumar V, Cheng SC, Johnson MD, et al. Immunochip SNP array identifies novel genetic variants conferring susceptibility to candidaemia. Nat Commun **2014**; 5:4675.
14. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience **2015**; 4:7.
15. Matzaraki V, Gresnigt MS, Jaeger M, et al. An integrative genomics approach identifies novel pathways that influence candidaemia susceptibility. PLoS One **2017**; 12:e0180824.
16. Lamparter D, Marbach D, Rueedi R, Kutalik Z, Bergmann S. Fast and rigorous computation of gene and pathway scores from SNP-based summary statistics. PLoS Comput Biol **2016**; 12:e1004714.
17. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet **2016**; 48:1279–83.
18. Võsa U, Claringbould A, Westra HJ, et al. Unraveling the polygenic architecture of complex traits using blood eQTL meta-analysis. bioRxiv **2018**; 447367.
19. Smeekens SP, Ng A, Kumar V, et al. Functional genomics identifies type I interferon pathway as central for host defense against *Candida albicans* Sanne. Nat Commun **2013**; 4:1–18.
20. Liu L, Okada S, Kong XF, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J Exp Med **2014**; 8:1635–48.
21. van de Veerdonk FL, Plantinga TS, Hoischen A, et al. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med **2011**; 365:54–61.
22. Song C, Chang XJ, Bean KM, Proia MS, Knopf JL, Kriz RW. Molecular characterization of cytosolic phospholipase A2-beta. J Biol Chem **1999**; 274:17063–7.

23. Jayaraja S, Dakhama A, Yun B, et al. Cytosolic phospholipase A2 contributes to innate immune defense against *Candida albicans* lung infection. BMC Immunol **2016**; 17:27.
24. Tallima H, El Ridi R. Arachidonic acid: physiological roles and potential health benefits - a review. J Adv Res **2018**; 11:33–41.
25. Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. Nutr Metab (Lond) **2011**; 8:36.
26. Lemmon MA. Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol **2008**; 9:99–111.
27. Martin TF. Phosphoinositide lipids as signaling molecules: common themes for signal transduction, cytoskeletal regulation, and membrane trafficking. Annu Rev Cell Dev Biol **1998**; 14:231–64.
28. Hubler MJ, Kennedy AJ. Role of lipids in the metabolism and activation of immune cells. J Nutr Biochem **2016**; 34:1–7.
29. Kominsky DJ, Campbell EL, Colgan SP. Metabolic shifts in immunity and inflammation. J Immunol **2010**; 184:4062–8.
30. Khovidhunkit W, Kim MS, Memon RA, et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res **2004**; 45:1169–96.
31. Aratani Y, Kura F, Watanabe H, et al. Critical role of myeloperoxidase and nicotinamide adenine dinucleotide phosphate-oxidase in high-burden systemic infection of mice with *Candida albicans*. J Infect Dis **2002**; 185:1833–7.
32. Frohner IE, Bourgeois C, Yatsyk K, Majer O, Kuchler K. *Candida albicans* cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. Mol Microbiol **2009**; 71:240–52.
33. Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. Nat Rev Genet **2012**; 13:175–88.
34. Long JZ, Cravatt BF. The metabolic serine hydrolases and their functions in mammalian physiology and disease. Chem Rev **2011**; 111:6022–63.
35. Wang T, Xu C, Zhou X, et al. Homozygous ALOXE3 nonsense variant identified in a patient with non-bullous congenital ichthyosiform erythroderma complicated by superimposed bullous Majocchi's granuloma: the consequences of skin barrier dysfunction. Int J Mol Sci **2015**; 16:21791–801.
36. Dimeloe S, Burgener AV, Grählert J, Hess C. T-cell metabolism governing activation, proliferation and differentiation; a modular view. Immunology **2017**; 150:35–44.
37. Lachmandas E, Boutens L, Ratter JM, et al. Microbial stimulation of different Toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. Nat Microbiol **2016**; 2:16246.
38. Netea MG, Dinarello CA. More than inflammation: interleukin-1β polymorphisms and the lipid metabolism. J Clin Endocrinol Metab **2011**; 96:1279–81.
39. Netea MG, Demacker PN, de Bont N, et al. Hyperlipoproteinemia enhances susceptibility to acute disseminated *Candida albicans* infection in low-density-lipoprotein-receptor-deficient mice. Infect Immun **1997**; 65:2663–7.
40. Netea MG, Curfs JH, Demacker PN, Meis JF, Van der Meer JW, Kullberg BJ. Infusion of lipoproteins into volunteers enhances the growth of *Candida albicans*. Clin Infect Dis **1999**; 28:1148–51.
41. Suzuki YJ, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. Free Radic Biol Med **1996**; 22:269–85.
42. Pompéia C, Cury-Boaventura MF, Curi R. Arachidonic acid triggers an oxidative burst in leukocytes. Braz J Med Biol Res **2003**; 36:1549–60.
43. Ben-Ari J, Wolach O, Gavrieli R, Wolach B. Infections associated with chronic granulomatous disease: linking genetics to phenotypic expression. Expert Rev Anti Infect Ther **2012**; 10:881–94.
44. Fairfax BP, Makino S, Radhakrishnan J, et al. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet **2012**; 44:502–10.
45. Brown CD, Mangravite LM, Engelhardt BE. Integrative modeling of eQTLs and cis-regulatory elements suggests mechanisms underlying cell type specificity of eQTLs. PLoS Genet **2013**; 9:e1003649.
46. Zeller T, Wild P, Szymczak S, et al. Genetics and beyond–the transcriptome of human monocytes and disease susceptibility. PLoS One **2010**; 5:e10693.
47. GTEx Consortium. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science **2015**; 348:648–60.
48. Westra HJ, Peters MJ, Esko T, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet **2013**; 45:1238–43.
49. Fairfax BP, Humburg P, Makino S, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science **2014**; 343:1246949.